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Eppendorf Reference® 2

Operating manual

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Further information can be found on the enclosed CD.

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1 Operating instructions

1.1 Using this manual

- ▶ Read this operating manual completely before using the device for the first time. Please also note the operating instructions for the accessories, if applicable.
- ▶ This operating manual is part of the product. Thus, it must always be easily accessible.
- ▶ Enclose this operating manual when transferring the device to third parties.

1.2 Danger symbols and danger levels

The safety instructions in this manual appear with the following danger symbols and danger levels:

1.2.1 Danger symbols

1.2.2 Danger levels

DANGER	<i>Will</i> lead to severe injuries or death.
WARNING	<i>May</i> lead to severe injuries or death.
CAUTION	May lead to light to moderate injuries.
NOTICE	May lead to material damage.

1.3 Symbols used

Representation	Meaning
▶	Handling
1. 2.	Actions in the specified order
•	List
→	Direction of movement
<i>Text</i>	Name of fields in the software
ⓘ	Useful information

2 Product description

2.1 Features

Reference 2 pipettes are piston-stroke pipettes which operate according to the air-cushion principle. For air cushion pipettes, a suitable pipette tip must be fitted prior to use. The user controls the dispensing, volume setting and ejector using the control button. When the control button is pressed, the piston in the pipette moves in the same direction. If the control button and the piston are moved upward, the liquid can be aspirated into the pipette tip. When the piston is moved downward, the liquid is dispensed (measuring stroke). Blow-out occurs if the control button is pressed down past the first stop. If the control button is pressed down again, the pipette tip will be ejected. The tip cone will spring load when a pipette tip is attached (exceptions: 2 mL to 10 mL pipettes).

The Reference 2 pipettes can be adjusted. A viewing window below the adjustment seal indicates that a user adjustment has been modified. The user adjustment functions independent of the factory adjustment.

All Reference 2 pipettes are fully autoclavable.

2.1.1 Pipette models

The Reference 2 family consists of single-channel and multi-channel pipettes (8 and 12-channels) with variable volume settings, and single-channel fixed-volume pipettes. For Reference 2 pipettes with variable volume settings, the volume can be changed by turning the control button when the volume locking button is pressed. Volumes from 0.1 µL to 10 mL can be dispensed depending on the model.

A list of all available models is available in the Technical Data.

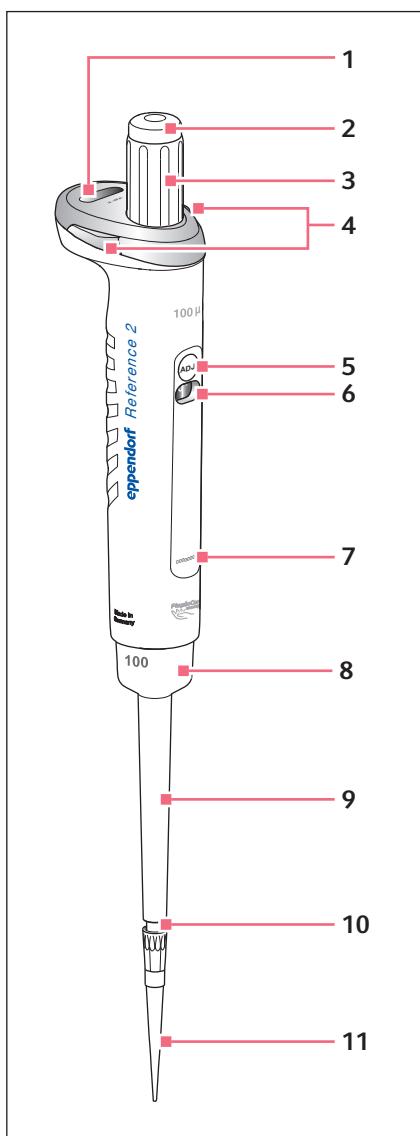
2.2 Delivery package

The following accessories are included in the delivery package:

Piece	Description
1	Operating manual
1	Certificate
1	Allen key with a blue handle (adjustment tool)
1	CD with information on chemical resistance, user adjustment, factory adjustment and ordering information
1	Black locking ring for single-channel pipettes ≤ 1000 µL
5	Red plastic adjustment seal (ADJ)
5	Protection filter consisting of 1 filter sleeve and 10 filter cartridges (for 2 mL to 10 mL pipettes)
1	Pipette wrench for opening the lower part (2 mL to 10 mL)
1	Multi-channel tool for cutting the O-rings (only 100 µL and 300 µL multi-channel lower parts)
5	Red plastic safety plug
1	Pin

2.3 Main illustration

2.3.1 Reference 2 single-channel



1 Volume display

Volume display with four-digit counter for variable pipettes. Decimal places are marked by a hyphen.

2 Color code

The color code on the control button and the trays of the matching epT.I.P.S. pipette tips are in the same color.

3 Control button

Sets and dispenses the volume, and ejects the pipette tip.

4 Volume locking button

Locks the control button volume setting ring. Press a volume locking button at the left or right to set the volume.

5 User adjustment

Upon delivery, the opening is sealed using a gray plastic adjustment seal (ADJ).

6 User adjustment display

Set to "0" before delivery.

7 Serial number

Serial number for upper and lower parts.

8 Nominal volume

9 Ejector sleeve

Ejects the pipette tip. Press the control button down all the way.

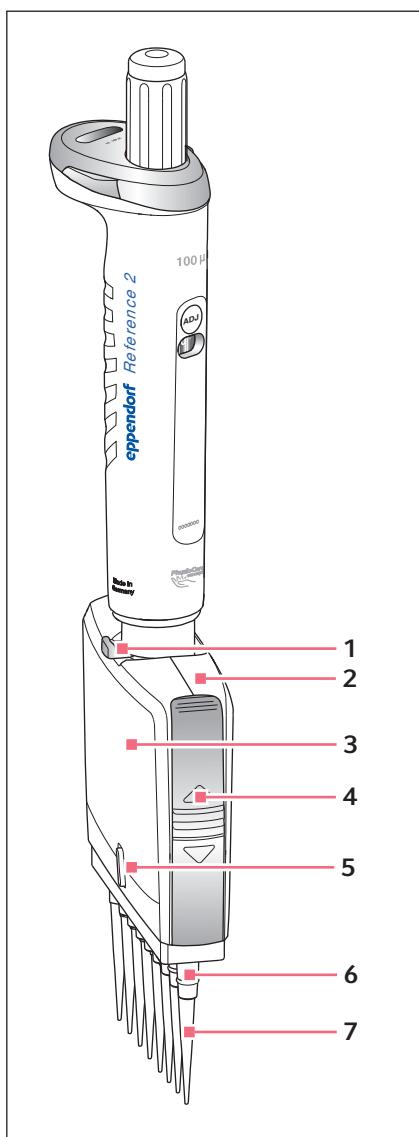
10 Spring-loaded tip cone

The spring-loading action optimizes the force required to attach and eject tips (no spring-loaded action for 2 mL to 10 mL). For 2 mL to 10 mL pipettes, an exchangeable protection filter in a sleeve is located in the tip cone.

11 Pipette tip

We recommend using epT.I.P.S.

2.3.2 Reference 2 Multi-channel



For an explanation of the upper part of the pipette, refer to the main illustration of the single-channel pipette.

1 Lever

Loosens the multi-channel lower part.

2 Housing lid

Removable housing lid with retracted ejector rail.

3 Multi-channel lower part

The multi-channel lower part is freely rotatable. The lower part does not detach when it is rotated. The outer channels are labeled with the numbers 1 and 8 (or 12).

The multi-channel version has a piston for each channel to ensure that a smaller number of pipette tips can also be mounted.

The lower part can be converted from an 8 to a 12-channel version with the same volume range. The multi-channel lower part can be opened, enabling you to replace or remove individual channels.

4 Latches

One right latch and one left latch to unlock the housing lid.

5 Selection dial On/Off

Switches on or off the spring loading of the tip cones.

6 Tip cones

Spring-loaded tip cones optimize the force required to attach and eject pipette tips.

7 Pipette tips

We recommend using epT.I.P.S.

2.4 Materials



NOTICE! Aggressive substances may damage the Reference 2 and accessories.

- ▶ Check the chemical resistance before using organic solvents or aggressive chemicals.
- ▶ Please note the cleaning instructions.
- ▶ You can find further information on chemical resistance on the included CD or on our webpage www.eppendorf.com.

The Reference 2 components that can be accessed by the user are made of the following materials:

Component	Material
External surfaces of the upper part	<ul style="list-style-type: none">• Improved polypropylene (PP)• Polycarbonate (PC)• Polyetherimide (PEI)• Stainless steel
Exterior and interior of lower parts	<ul style="list-style-type: none">• Improved polypropylene (PP)• Polyvinylidene fluoride (PVDF)• Polyetherimide (PEI)• Polyphenylene sulfide (PPS)• Polyetheretherketone (PEEK)• Polytetrafluoroethylene (PTFE)• Ethylene propylene diene rubber (EPDM)• Silicone• Steel (stainless steel and spring steel)

Pipette tip	Material
epT.I.P.S.	Polypropylene (PP)
epDualfilter T.I.P.S. filter	Polyethylene (PE)

2.5 Storing the pipette

The pipette can be safely stored in a pipette carousel or a wall mounting device.

2.5.1 Pipette carousel

A pipette carousel (4923) is available for storing Reference 2 pipettes. The pipette carousel features 6 holders that are designed for the Reference 2. Each holder features an image of a Reference 2 pipette to ensure that the pipettes are placed in the correct holder.

2.5.2 Holder

The holder can also be installed in the pipette carousel (3115) for Research plus pipettes.

2.5.3 Wall mount

An adhesive strip is used to prepare the wall mount for assembly on a level surface (glass, tile).

3 Safety

3.1 Intended use



The intended use is described in a separate document enclosed in the package.

3.2 Warnings for intended use



WARNING! Damages to health due to infectious liquids and pathogenic germs.

- ▶ When handling infectious liquids and pathogenic germs, observe the national regulations, the biological security level of your laboratory, the material safety data sheets, and the manufacturer's application notes.
- ▶ Wear personal protective equipment.
- ▶ Consult the "Laboratory Biosafety Manual" (Source: World Health Organization, Laboratory Biosafety Manual, as amended) for comprehensive regulations on the handling of risk group II germs or biological materials.



WARNING! Damage to health due to toxic, radioactive or aggressive chemicals.

- ▶ Wear personal protective equipment.
- ▶ Observe the national regulations for handling these substances.
- ▶ Observe the material safety data sheets and manufacturer's application notes.



CAUTION! Danger to individuals due to careless use.

- ▶ Never point the opening of a Reference 2 which is equipped with a pipette tip at yourself or other persons.
- ▶ Only initiate dispensing if it is safe to do so.
- ▶ With any dispensing task please ensure that you do not endanger yourself and other persons.



CAUTION! Poor safety due to incorrect accessories and spare parts.

The use of accessories and spare parts other than those recommended by Eppendorf may impair the safety, functioning and precision of the device. Eppendorf cannot be held liable or accept any liability for damage resulting from the use of incorrect or non-recommended accessories and spare parts, or from the improper use of such equipment.

- ▶ Only use accessories and original spare parts recommended by Eppendorf.

**NOTICE! Damage to device from missing pipette tips.**

- ▶ Use the Reference 2 with fitted pipette tips only.
- ▶ When using pipette tips without filters, use 2-10 mL pipettes with protective sleeves and an inserted protection filter.

**NOTICE! Carry-over, contamination and incorrect dispensing results due to the incorrect use of pipette tips.**

The pipette tips are for single use only. Prolonged use can have a negative impact on dispensing tasks.

- ▶ Only use the pipette tips once.
- ▶ Do not use autoclaved ep Dualfilter T.I.P.S. for dispensing.

**NOTICE! Incorrect dispensing volume due to differences in temperature and for special liquids.**

Temperature differences between the pipette, pipette tips and liquid; or solutions with physical data that is very different from that of water; may result in incorrect dispensing volumes.

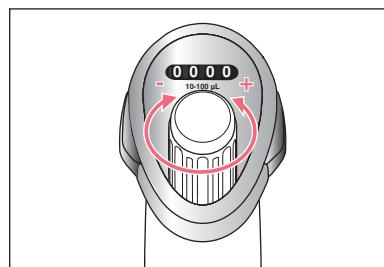
- ▶ Avoid temperature differences between the pipette, pipette tip and liquid.
- ▶ Ensure that the temperature is between 20 °C and 27 °C and remains constant at ± 0.5 °C.
- ▶ Check the dispensing volume and make sure that you can affirm all the questions listed in the general information.

**NOTICE! Damage to the device due to penetration of liquids.**

- ▶ Do not put the pipette down when the pipette tip is filled.
- ▶ Do not allow any liquids to penetrate the inside of the housing.
- ▶ If liquid has entered the inside of the housing, the inner parts may only be repaired by Eppendorf AG service partners. Contact your local sales office before returning any devices.

4 Operation

4.1 Setting the volume (only Reference 2 variable)



- ▶ To set the volume, hold the volume locking button pressed down and turn the control button.

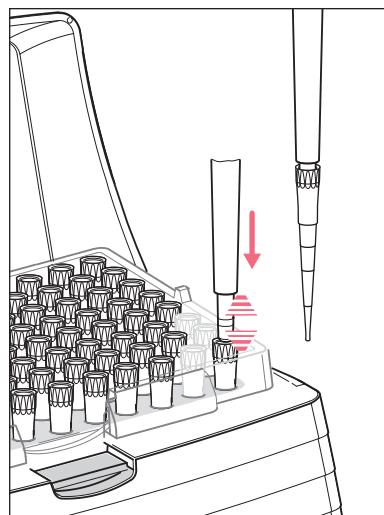
We recommend adjusting the volume setting from a higher value to a lower value. If required, turn beyond the required value and then back again.

The height of the control button changes as the volume is adjusted. The volume is displayed up to 1000 µL in µL. For 2 mL to 10 mL pipettes, the volume is displayed in mL.

4.2 Using pipette tips

The liquid to be dispensed is aspirated into pipette tips. We recommend using epT.I.P.S. epT.I.P.S. pipette tips, which are available with various degrees of purity, with and without filters and as special tips. We recommend using special epT.I.P.S. LoRetention tips with liquids that have a lower surface tension than water (e.g., due to a wetting agent in the liquid).

Pipette tips are for single use only.



The pipette is only functional if a pipette tip has been attached. You can either attach the pipette tip by hand or directly insert the end of the pipette into a tip held in the tip storage box. If attaching a pipette tip by hand, it must be handled in a way that prevents contamination and heating of the pipette tip.

The color code on the Reference 2 control button matches the color of the epT.I.P.S. trays

- ▶ If you use pipette tips without filter, insert the protection filter in the 2 mL to 10 mL pipette.
- ▶ Fit the suitable pipette tip(s) on the tip cone, applying light pressure.

The pipette tip sits firmly on the tip cone when it spring loads (exceptions: no spring loading for 2 mL to 10 mL single-channel pipettes.)

4.3 Deactivating the tip cone spring loading

For the following dispensing tasks, it may be helpful to deactivate the spring loading action of the tip cone.

- A pipette tip is to be used over a long period of time.
- The pipette tip is slightly bent during dispensing.
- For pipette tips that are not designed for the tip cone of Eppendorf pipettes.

The spring loading action of the tip cone on single-channel pipettes can be blocked by installing a locking ring.

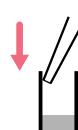
On multi-channel pipettes, the spring loading action of the tip cone can be switched on or off using the selection switch.

4.4 Aspirating liquid



1. The liquid to be aspirated must be taken from a suitable vessel. For multi-channel pipettes, we recommend the reagent reservoir "Tip-Tub".
2. Press down the control button to the first stop (measuring stroke).
3. Immerse the pipette tip(s) vertically approx. 4 mm into the liquid.
4. To aspirate liquid, allow the control button to slide back slowly. Maintain the immersion depth to ensure no air is aspirated.
5. In the case of large volumes: before removing the pipette tip from the liquid, wait for approx. 3 seconds. To ensure maximum precision and accuracy, we recommend to wet each new tip initially by aspirating and dispensing the liquid one to three times. Only then should pipetting commence.
6. Remove the tip(s) slowly from the liquid.
7. Wipe the tip(s) slowly against the tube wall to ensure that no outer wetting remains on the tip.

4.5 Dispensing liquid



1. Place the tip on the tube inner wall at an angle.
2. Press the control button slowly until the first stop (measuring stroke) and wait until the flow of liquid stops.
3. To empty the tip, press the control button until the second stop (blow-out).
4. Hold down the control button and wipe the tip on the tube inner wall.
5. To eject the tips, hold the control button all the way down.

5 Troubleshooting

5.1 Error search

Symptom/message	Cause	Remedy
Liquid is dripping from the tip and/or the dispensed volume is incorrect.	The tip is loose or the pipette tip is poorly fitted.	► Firmly attach the pipette tips. Use epT.I.P.S. pipette tips.
	Liquid with high vapor pressure and/or different density.	► Wet the tip several times and adjust the pipette for the liquid used.
	Pipetting was too quick.	► Move the control button slowly.
	The tip is withdrawn from the liquid too quickly.	► Withdraw the tip slowly from the liquid with a time delay (approx. 3 seconds).
	Liquid aspirated with blow-out and dispensed with blow-out.	► Repeat dispensing correctly.
	Piston dirty or damaged.	► Clean piston, grease slightly and/or replace.
	The tip cone is damaged.	► Replace the lower part or channel.
The control button jams and does not move smoothly.	The O-rings of the tip cones are damaged.	► Replace the O-rings (only 100 µL, 300 µL multi-channel).
	<ul style="list-style-type: none">• The piston is dirty.• The seal is dirty.• Seal defective.• The pipette is blocked.	<ul style="list-style-type: none">► Clean the lower part.► Replace the protection filter on 2 mL to 10 mL models.
The adjustment seal has been removed; the adjustment display has been changed.	The pipette has been adjusted for a different liquid.	► Adjust the pipette for the liquid used.
No spring-loading action of the tip cone when pipette tips are inserted.	Spring-loading action is blocked by a locking ring. Spring loading is switched off (multi-channel).	<ul style="list-style-type: none">► Remove the locking ring again.► Switch on spring loading.
	Use of a 2 mL to 10 mL pipette.	No remedy available. The tip cone of these sizes does not have spring-loading action.

Regularly check the precision and accuracy of the Reference 2 to prevent dispensing errors. You can use the "PICASO" software to determine the maximum permissible systematic and random errors. If the pipette is used at an extremely high altitude, it must be adjusted to the ambient air pressure. A Standard Operating Procedure (SOP) for checking pipettes can be found on the Reference 2 CD and on our webpage www.eppendorf.com.

6 Maintenance



NOTICE! Aggressive substances may damage the Reference 2 and accessories.

- ▶ Check the chemical resistance before using organic solvents or aggressive chemicals.
- ▶ Please note the cleaning instructions.
- ▶ You can find further information on chemical resistance on the included CD or on our webpage www.eppendorf.com.

- All single and multi-channel lower parts are wear parts. Clean them after contamination, use of aggressive chemicals and/or heavy stress. If the lower parts are worn or damaged, replace these parts.
- Faulty dispensing results are sometimes due to lack of maintenance.

6.1 Cleaning



NOTICE! Damage to device from unsuitable cleaning fluids or sharp or pointed objects.

Unsuitable cleaning fluids can damage the device.

- ▶ Never use corrosive cleaning fluids, strong solvents or abrasive polishes.
- ▶ Check the compatibility with the materials used.
- ▶ Please note the information on chemical resistance (see CD).
- ▶ Do **not** clean the Reference 2 with acetone or organic solvents with a similar effect.
- ▶ Do **not** clean the Reference 2 with sharp objects.

Remove external contamination on the Reference 2 as follows:

- ▶ Wet a cloth with a mild cleaning fluid and water and remove the contamination.
- ▶ To remove heavy contamination resulting from liquid penetration, disassemble the lower part of the pipette and clean it with demineralized water.

Relubricate the piston sealing rings after contamination, use of aggressive chemicals and/or heavy stress. Remove the old grease before relubricating.

Only use the grease specified in the ordering information (see the enclosed CD).

6.2 Sterilizing or disinfecting the pipette



NOTICE! Damage to device from incorrect handling.

- ▶ Do not use any additional disinfectants, decontamination agents or sodium hypochlorite during autoclaving or UV exposure.
- ▶ When autoclaving, please ensure that the temperature does not exceed 121°C.
- ▶ Before using disinfecting agents or decontaminating agents, test for material compatibility and check the manufacturer's instructions on chemical resistance. Please also note the pipette material.

6.2.1 Autoclaving

All Reference 2 pipettes are completely steam autoclavable.

6.2.1.1 Before autoclaving

- ▶ Remove any contamination from the exterior and the lower part.
- ▶ If you remove existing grease, slightly re-lubricate the piston seal using only the grease specified in the ordering information (see the enclosed CD).

6.2.1.2 Procedure

Prerequisites

- Follow the operating manual of the autoclave manufacturer.
- Autoclave at: 121 °C, 20 minutes, 1 bar overpressure.

1. When placing the Reference 2 into the autoclave, make sure that the temperature at the pipette does not exceed 121 °C.
2. You can put the Reference 2 into the autoclave as a complete unit or with the lower part removed. Do not disassemble the lower part.
3. For 2 mL to 10 mL pipettes: remove old protection filter. Add a filter sleeve and new protection filter and insert them after autoclaving. Only autoclave the protection filter once.
4. If lower parts have been removed, make sure that no lower parts are confused during reassembly. (Tip: use one plastic beaker per pipette.)

6.2.1.3 After autoclaving

- ▶ Cool the pipette to room temperature and let it dry.
- ▶ For 2 mL to 10 mL pipettes: press the protection filter into the filter sleeve and insert it into the tip cone. The piston does not need to be relubricated after autoclaving. If autoclaving is carried out as described above, no gravimetric testing or adjustment is necessary.

6.2.2 Disinfection

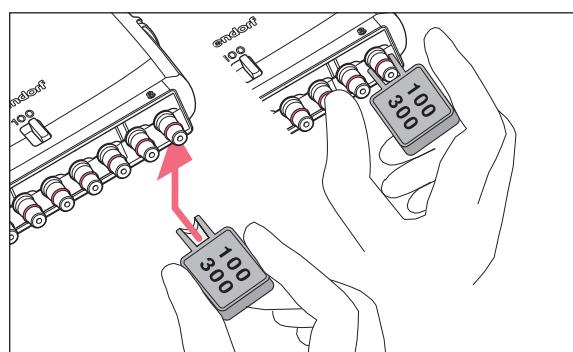
- ▶ Carefully wipe off the outer surfaces with disinfectant, DNA/RNA decontamination agents or 70 % isopropanol.
- ▶ You can wipe the outside of the tip cone and the ejector sleeve using a sodium hypochlorite solution of 4 %.
- ▶ After the exposure time of the sodium hypochlorite solution (15 minutes) has elapsed, thoroughly remove it using demineralized water.

The Reference 2 pipettes may be temporarily subjected to the UV light of a sterile bench (≥ 254 nm).

6.3 Replacing O-rings

The 100 μL and 300 μL multi-channel lower parts are equipped with O-rings. These are wear parts. Replace old, worn or damaged O-rings. Defective O-rings result in the incorrect positioning of the tips and in dispensing errors.

6.3.1 Removing the O-ring



1. Switch off the spring loading.
2. Push the opening of the O-ring tool (included in the delivery package) against the tip cone from the side so that the sharp edge in the opening of the O-ring tool is positioned on top of the o-ring. Do not slide the O-ring tool onto the cone!
3. Supporting the O-ring tool with your thumb, push it firmly against the tip cone. This O-ring is cut in one place.
4. Remove the O-ring tool and the O-ring from the tip cone.

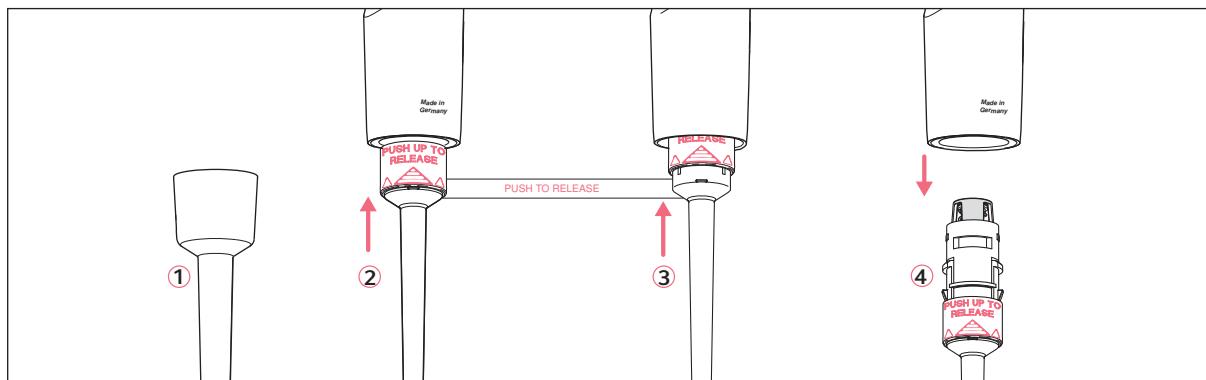
6.3.2 Mounting a new O-ring

1. Fit the mounting aid (shortened pipette tip, included in the delivery package of the O-rings) on the tip cone.
2. Push the new O-ring over the tip onto the tip cone.
3. Check that the pipette tips are correctly positioned. Ensure that the tips are tightly sealed and properly aligned.

6.4 Disassembling and assembling the pipette lower part

6.4.1 Single-channel lower part up to 1000 µL

6.4.1.1 Removing the lower part



1. Hold the control button completely pressed down.
2. Remove the ejector sleeve ①.
3. ② and ③ : On the lower part, slide the ring marked "PUSH UP TO RELEASE" up by about 5 mm until the lower part comes off.
4. ④ : Remove the lower part from the upper part.

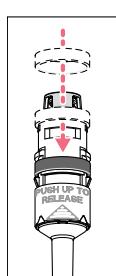
6.4.1.2 Deactivating spring loading

For the following dispensing tasks, it may be helpful to deactivate the spring loading action of the tip cone.

- A pipette tip is to be used over a long period of time.
- The pipette tip is slightly bent during dispensing.

Deactivating this action may also be helpful for pipette tips that are not designed for the Eppendorf pipette tip cone.

6.4.1.2.1 Single-channel pipettes



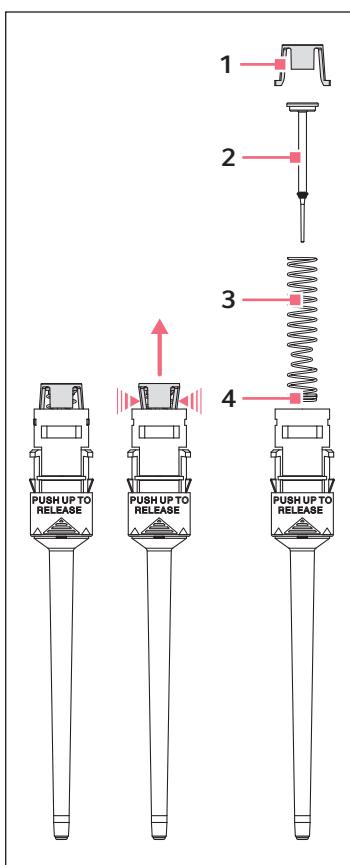
The spring loading action of the tip cone can be deactivated by installing the locking ring. The locking ring is included in the delivery package.

1. Push the black locking ring onto the lower part from the top by slightly squeezing the clamps on the lower part.
2. Insert the lower part into the upper part until it audibly engages.
3. Hold the control button completely pressed down.
4. Mount the ejector sleeve. If correctly fitted, it will engage slightly.
Unlock the locking ring to reactivate the spring-loading action.

6.4.1.3 Opening and assembling the lower part ($\leq 1000 \mu\text{L}$)



To avoid confusion of parts, only disassemble and assemble one pipette at a time.



Opening the lower part:

1. Push the stop pins together slightly on the piston mount (1).
2. Remove the piston mount.
3. Remove the piston (2) and the piston spring (3). The piston is under spring tension. The appearance of pistons and piston springs will vary for the individual volume versions.

Assembling the lower part:

1. Carefully guide the piston and the piston spring into the cylinder. Make sure that the piston is guided correctly in the piston spring and the cylinder. There must not be any perceptible resistance. **Stop pushing as soon as any resistance is felt.** This may indicate that the piston is not positioned correctly in the cylinder. There is a risk of bending the piston if too much force is applied. Carefully pull out the piston and repeat the process correctly. In the case of piston springs with double windings (4), these windings must point downwards.
2. Keep the piston and the piston spring pressed.
3. Keep the stop pins on the piston mount pressed with the other hand.
4. Mount the piston mount so that the two locking mechanisms engage in their retainers.
5. Gently press a pipette tip against the inserted piston. The piston must move down in the cylinder without any noticeable resistance.

Installation of the lower part and ejector sleeve:

1. Insert the lower part into the upper part until it audibly engages.
2. Set the maximum volume and operate the control button several times. It must run smoothly and resistance-free.
3. Keep the ejector pressed. The ejector rod protrudes from the upper part.
4. Place the ejector sleeve on the ejector rod. If correctly fitted, it will engage slightly.
5. Carry out a gravimetric test of the systematic and random error. This test ensures that no parts were confused during assembly and that the pipette has been assembled correctly.

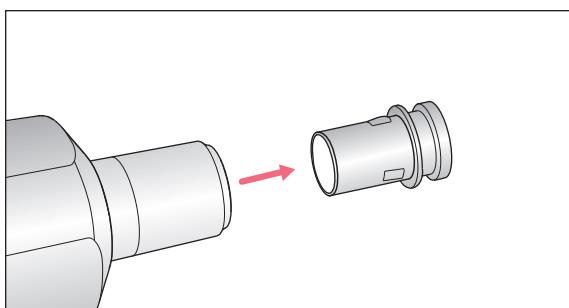
6.4.2 Single-channel lower part

Applies to the following single-channel pipettes:

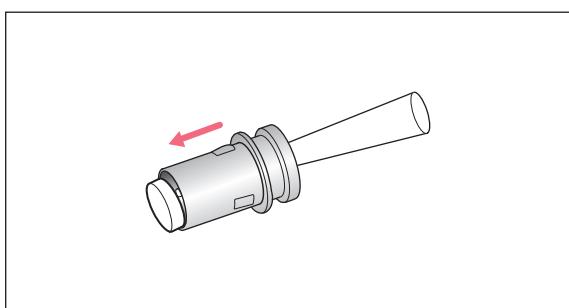
- 2 mL
- 2.5 mL
- 5 mL
- 10 mL

Replace the protection filter in the tip cone each time it comes into contact with liquid.

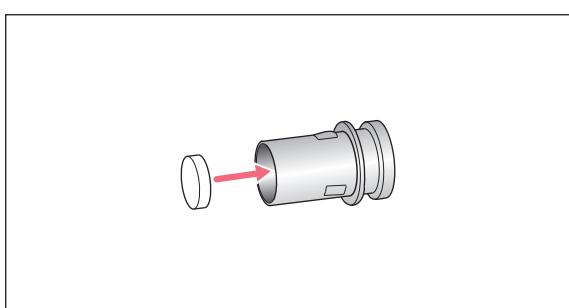
6.4.2.1 Exchanging the filter



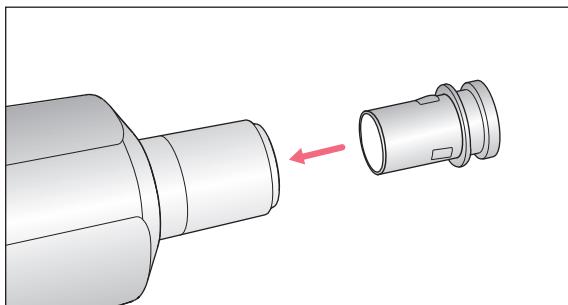
1. Remove the filter sleeve.



2. Use a pipette tip to push out the old filter.

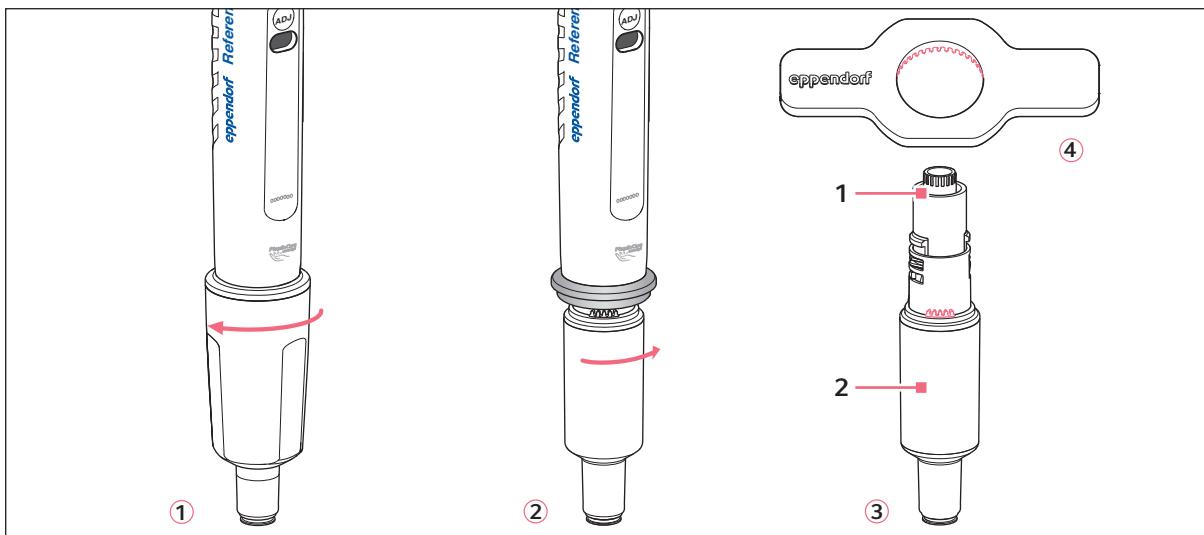


3. Insert a new filter into the filter sleeve.



4. Insert the filter sleeve.

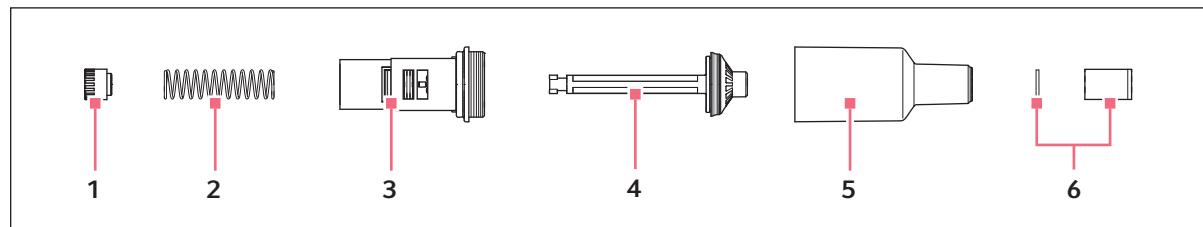
6.4.2.2 Opening and assembling the lower part (2 mL or larger)



1. ① : Unscrew the ejector sleeve.
2. ② : Keep the control button fully pressed and turn the lower part approximately 30° to the right.
The lower part is automatically released from the upper part.
3. ③ : Open the lower part: fit the pipette key ④ (included in the delivery package) onto the lower part. Hold the cylinder and unscrew it from the lower part.

See the next figure.

The lower part consists of the following:



- | | |
|---------------------------------------|---|
| 1 2 mL – 10 mL piston mount | 4 Piston (with seal) |
| 2 Piston spring | 5 Cylinder and tip cone |
| 3 Upper part of the lower part | 6 Filter sleeve with protection filter in the tip cone |

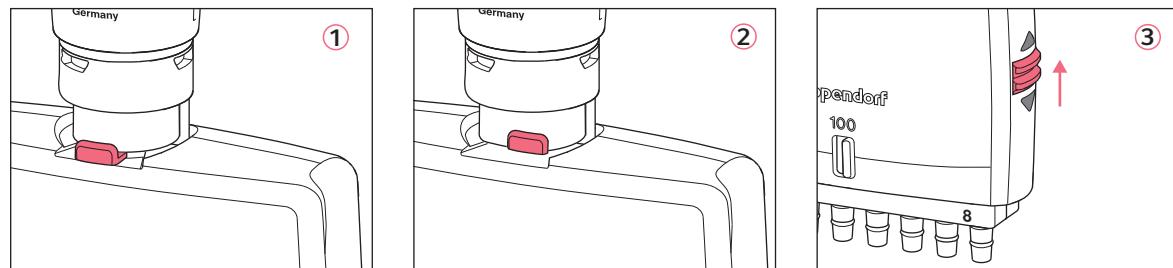
Before assembling the pipette, first install the piston mount, the piston spring, and the piston in the upper part of the lower part. Further assembly is as shown in the figures. Carry out the procedure in reverse order.

- ▶ Carry out a gravimetric test of the systematic and random errors.

This test ensures that no parts were confused during assembly and that the pipette has been assembled correctly.

6.4.3 Multi-channel lower part

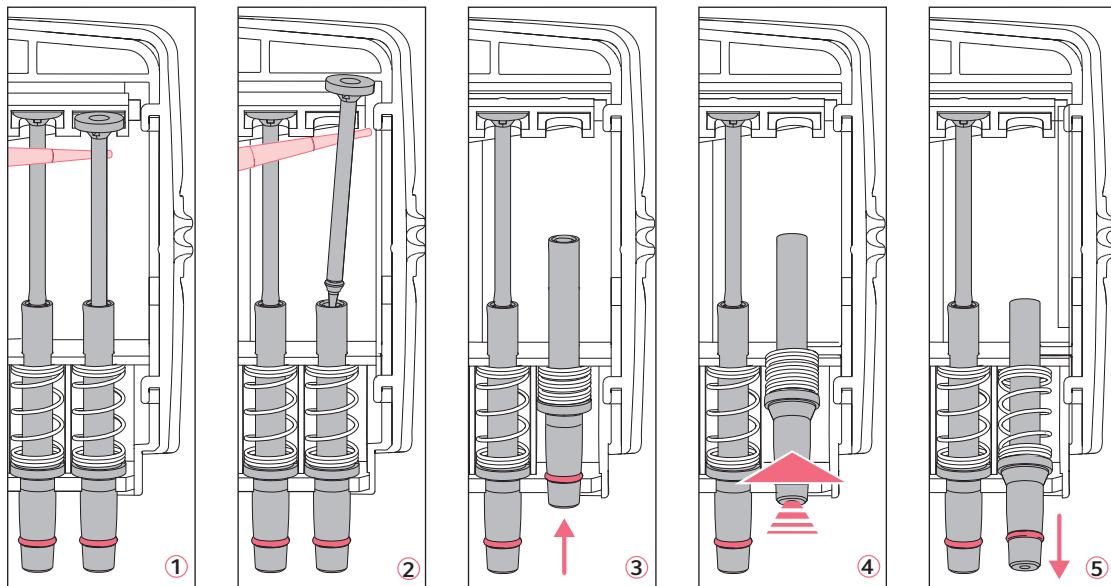
6.4.3.1 Loosen and open the 10 µL, 100 µL and 300 µL multi-channel lower part



1. Set the selection dial for spring loading to **On**.
2. ① and ② : Slide the lever on the lower part to the left or right.
This separates the lower part from the upper part so that it can be removed.
3. Put down the lower part with the lever facing downwards.
4. ③ : Slide the two latches (right and left at the side) up.
The lower part is still lying on the table with the lever facing downwards.
5. Remove the cover plate facing upward and the integrated ejector rail.

Do **not** remove the ejector rail from the cover plate. The spring for the ejector rail may accidentally come off and get lost during this step.

6.4.3.2 Removing and installing channels



1. ① and ② : Position a pipette tip under the piston and carefully remove the piston from the upper rail.
2. Carefully pull the piston out in an upward direction. **Do not bend the piston.**
3. Hold the tip cone at the lower end and push it upwards slightly ③.
This compresses the spring.
4. ④ : Lightly lift the tip cone and release it from the lower rail.
5. ⑤ : Relax the spring by letting the tip cone slide back above the lower rail.
6. Remove the tip cone, along with the cylinder and spring, from the upper rail.

Before installation, slide the piston into the cylinder. Install the channels in reverse order.

6.4.3.3 Assembling the 10 – 300 µL multi-channel pipette

1. Fit the cover plate with the integrated ejector.
2. Push the latches downward.
3. To mount, push the lower part into the upper part until it audibly engages.
4. Carry out a gravimetric test of the systematic and random error.
This test ensures that no parts were confused during assembly and that the pipette has been assembled correctly.

6.5 Adjusting pipettes



NOTICE! Incorrect dispensing volume due to differences in temperature and for special liquids.

Temperature differences between the pipette, pipette tips and liquid; or solutions with physical data that is very different from that of water; may result in incorrect dispensing volumes.

- ▶ Avoid temperature differences between pipette, pipette tip and liquid.
- ▶ Ensure that the temperature is between 20 °C and 27 °C and remains constant at ± 0.5 °C.
- ▶ Check the dispensing volume and make sure that you can affirm all the questions listed in the general information.



The random and systematic errors recorded on delivery can be found in the *Eppendorf Certificate*. This certificate is included in delivery. Changes to the factory adjustment will render the certificate void.

6.5.1 General instructions on user and factory adjustment

The Reference 2 was adjusted and tested prior to delivery. The adjustment opening is sealed using a gray plastic adjustment seal. The adjustment seal is labeled with the abbreviation "ADJ". The adjustment display shows "0".

Changing the adjustment of the Reference 2 is sometimes recommended for solutions that are very different from water in terms of their density, viscosity, surface tension and/or vapor pressure, etc. If the density of an aqueous solution changes by approximately ± 10 %, for example, due to the salt concentration, the volume changes by approximately ± 0.2 %. This statement does not apply if other relevant properties of the liquid also change.

If the pipette is used at extremely high altitudes, it must be calibrated to the ambient air pressure. At 1000 m above sea level, the volume error of a 100 µL pipette is about -0.3 %.

When using special tips, i.e. tips with a geometry that is very different from standard tips, changing the adjustment can improve the dispensing accuracy (systematic error).

Follow the adjustment tips for epT.I.P.S. long provided in the *User adjustment* document on the Reference 2 CD.

Adjustment changes can be reset using simple steps.

In addition to changing the user adjustment, a Reference 2 can be permanently changed by altering the factory adjustment.

Changes to the user or factory adjustment do not affect the dispensing precision (random error). Precision can be improved by exchanging worn parts. Precision is also significantly affected by handling.

The existing dispensing volume must be checked before changing the factory calibration or adjustment.

The actual volume can be checked by weighing:

$$\text{Actual volume} = \frac{\text{Mean value of the weighings}}{\text{Density liquids at weighing temperature}}$$

The density of distilled water is approx. 0.9982 mg/ μL at 20 °C and 0.9965 mg/ μL at 27 °C.

If the set volume is the same as the actual volume, no correction is necessary.

If there is a difference between the actual volume and the set volume of distilled water, please check the following:

- Is there any liquid dripping from the tip?
- Is the pipette tip fitted leak-proof?
- Is the tip cone undamaged?
- Are the piston and cylinder leak-proof?
Adequate leak tightness is ensured when no drop forms on the pipette tip after aspiration of the nominal volume with distilled water and a waiting time of approx. 15 s. Hold the pipette vertically without touching the pipette tip. Prewet the tip several times for nominal volumes $\leq 20 \mu\text{L}$.
- Does the temperature of the pipetted liquid correspond to:
 - The temperature of the device?
 - The ambient air temperature?
- Is the weighing location free of drafts?
- Does the work method and pipetting speed permit complete aspiration and dispensing of the liquid?
- Has the correct numerical value for "Density liquids at weighing temperature" been used to calculate the actual volume?
- Is the set volume correct?
- For very small volumes ($< 10 \mu\text{L}$): Is the fine balance sufficiently sensitive (balance resolution: 0.001 mg)?
- Were original epT.I.P.S pipette tips used for testing?
Information on which pipette tip was used as the test tip can be found in the "Technical data" (see p. 33).

No adjustment changes are allowed unless you can answer all the questions with "yes". In all other cases, the problems associated with the questions answered with "no" must be eliminated. If the problem is remedied by exchanging a complete lower part or other parts that affect the volume, proper assembly must be verified by carrying out a gravimetric test. Information on the systematic and random errors to be met can be found in the "Technical data" chapter.

6.5.2 Changing the user adjustment

If the adjustment is changed, the volume changes by a certain value. Strictly speaking, the change only applies to the testing volume.

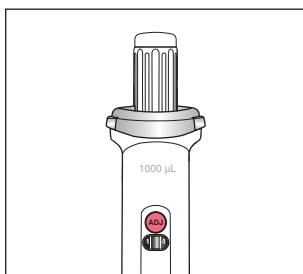
Example:

You readjust a 10 – 100 µL pipette with a volume setting of 100 µL by 1 µL ($1 \mu\text{L} \triangleq 1 \%$). If the volume setting is 10 µL, the pipette is also adjusted by 1 µL ($\triangleq 10 \%$).

Auxiliary aids from the delivery package

- Pin
- Red plastic adjustment seal (ADJ)

1. Insert the pin in the middle of the gray adjustment seal (ADJ).
2. Remove the adjustment seal.
3. Insert the adjustment tool.
4. Turn the adjustment tool until the adjustment display shows the desired value.
5. Read the set value distortion-free using the aligning aid in the viewing window.
6. Carry out weighings to check the accuracy and precision.



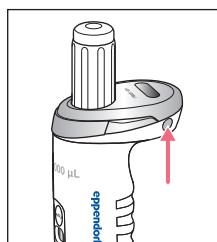
7. Attach the red adjustment seal after the inspections.

If the adjustment is meant for a specific liquid, mark the pipette accordingly. Use the labeling area on the pipette for this, and write down the liquid and the volume. Carry out a gravimetric test for each adjustment change. Follow the EN ISO 8655-2 and EN ISO 8655-6 test procedures. A Standard Operating Procedure (SOP) and further information on user and factory adjustment settings can be found on the Reference 2 CD and on our website www.eppendorf.com.

6.5.3 Changing the factory adjustment

Auxiliary aids from the delivery package

- Red plastic safety plug
- Pin



The factory adjustment can be changed using the corresponding accessories.

The factory adjustment will be marked by a safety plug. The color of the safety plug indicates the implementing authority:

- Gray - Eppendorf AG
- Red - User

The random and systematic errors recorded on delivery can be found in the *Eppendorf Certificate*. This certificate is included in delivery. The certificate is no longer valid if the default settings have been changed.

If the dispensing accuracy is only to be changed temporarily, the correct method is to change the user adjustment. Before changing the user or factory adjustment, observe the general notes (see *General instructions on user and factory adjustment on p. 28*) and the associated gravimetric tests.

The execution of changes to the factory adjustment is described on the Reference 2 CD. The parts required to complete the change are included in the delivery package and are listed in the ordering information on the Reference 2 CD.

6.6 Decontamination before shipment

If you wish to return the device to Eppendorf AG or an Eppendorf AG service partner to be checked or repaired, please note the following:



CAUTION! Use of a contaminated device may result in personal injuries and damage to the device.

- ▶ Clean and decontaminate the Reference 2 in accordance with the cleaning instructions before shipping or storage.

Hazardous substances are:

- solutions presenting a hazard to health
- potentially infectious agents
- organic solvents and reagents
- radioactive substances
- proteins presenting a hazard to health
- DNA

1. Please note the information in the document "Decontamination certificate for product returns".
You can find it as a PDF file on our webpage www.eppendorf.com.
The decontamination certificate can also be found on the enclosed CD.
2. Enter the serial number of the Reference 2 in the decontamination certificate.
3. With the shipment please include the completed decontamination certificate for product returns with the Reference 2.
4. Send the pipette to Eppendorf AG or an Eppendorf AG service partner. The address details of Eppendorf AG can be found on the last page of this operating manual. The list of service partners can be found at www.eppendorf.com/worldwide.

7 Technical data

7.1 Reference 2 Single-channel variable

Reference 2 single-channel variable

Model	Test tip epT.I.P.S. color code Volume range Length	Testing volume	Eppendorf AG error limits			
			Error			
			Systematic error		Random error	
			± %	± µL	± %	± µL
0.1 µL – 2.5 µL Increment: 0.002 µL	Dark gray 0.1 µL – 10 µL 34 mm	0.1 µL	±48.0	±0.048	±12.0	±0.012
		0.25 µL	±12.0	±0.03	±6.0	±0.015
		1.25 µL	±2.5	±0.031	±1.5	±0.019
		2.5 µL	±1.4	±0.035	±0.7	±0.018
0.5 µL – 10 µL Increment: 0.01 µL	Medium gray 0.1 µL – 20 µL 40 mm	0.5 µL	±8.0	±0.04	±5.0	±0.0025
		1 µL	±2.5	±0.025	±1.8	±0.018
		5 µL	±1.5	±0.075	±0.8	±0.04
		10 µL	±1.0	±0.10	±0.4	±0.04
2 µL – 20 µL Increment: 0.02 µL	Light gray 0.5 µL – 20 µL L 46 mm	2 µL	±5.0	±0.10	±1.5	±0.03
		10 µL	±1.2	±0.12	±0.6	±0.06
		20 µL	±1.0	±0.20	±0.3	±0.06
2 µL – 20 µL Increment: 0.02 µL	Yellow 2 µL – 200 µL 53 mm	2 µL	±5.0	±0.10	±1.5	±0.03
		10 µL	±1.2	±0.12	±0.6	±0.06
		20 µL	±1.0	±0.2	±0.3	±0.06
10 µL – 100 µL Increment: 0.1 µL	Yellow 2 µL – 200 µL 53 mm	10 µL	±3.0	±0.3	±0.7	±0.07
		50 µL	±1.0	±0.5	±0.3	±0.15
		100 µL	±0.8	±0.8	±0.2	±0.2
20 µL – 200 µL Increment: 0.2 µL	Yellow 2 µL – 200 µL 53 mm	20 µL	±2.5	±0.5	±0.7	±0.14
		100 µL	±1.0	±1.0	±0.3	±0.3
		200 µL	±0.6	±1.2	±0.2	±0.4
30 µL – 300 µL Increment: 0.2 µL	Orange 20 µL – 300 µL 55 mm	30 µL	±2.5	±0.75	±0.7	±0.21
		150 µL	±1.0	±1.5	±0.3	±0.45
		300 µL	±0.6	±1.8	±0.2	±0.6
100 µL – 1000 µL Increment: 1 µL	Blue 50 µL – 1000 µL 71 mm	100 µL	±3.0	±3.0	±0.6	±0.6
		500 µL	±1.0	±5.0	±0.2	±1.0
		1000 µL	±0.6	±6.0	±0.2	±2.0

Technical data

Eppendorf Reference® 2

English (EN)

Reference 2 single-channel variable

Model	Test tip epT.I.P.S. color code Volume range Length	Testing volume	Eppendorf AG error limits			
			Error			
			Systematic error		Random error	
			± %	± mL	± %	± mL
0.25 mL – 2.5 mL Increment: 0.002 mL	Red 0.25 mL – 2.5 mL 115 mm	0.25 mL	±4.8	±0.012	±1.2	±0.003
		1.25 mL	±0.8	±0.010	±0.2	±0.0025
		2.5 mL	±0.6	±0.015	±0.2	±0.005
0.5 mL – 5 mL Increment: 0.005 mL	Purple 0.1 mL – 5 mL 120 mm	0.5 mL	±2.4	±0.012	±0.6	±0.003
		2.5 mL	±1.2	±0.030	±0.25	±0.006
		5.0 mL	±0.6	±0.030	±0.15	±0.0075
1 mL – 10 mL Increment: 0.01 mL	Turquoise 1 mL – 10 mL 165 mm	1.0 mL	±3.0	±0.030	±0.6	±0.006
		5.0 mL	±0.8	±0.040	±0.2	±0.010
		10.0 mL	±0.6	±0.060	±0.15	±0.015

7.2 Reference 2 Multi-channel variable**Reference 2 Variable multi-channel (8-channel/12-channel)**

Model	Test tip epT.I.P.S. color code Volume range Length	Testing volume	Eppendorf AG error limits			
			Error			
			Systematic error		Random error	
			± %	± µL	± %	± µL
0.5 µL – 10 µL	Medium gray 0.1 µL – 20 µL 40 mm	0.5 µL	±12.0	±0.06	±8.0	±0.04
		1 µL	±8.0	±0.08	±5.0	±0.05
		5 µL	±4.0	±0.2	±2.0	±0.1
		10 µL	±2.0	±0.2	±1.0	±0.1
10 µL – 100 µL	Yellow 2 µL – 200 µL 53 mm	10 µL	±3.0	±0.3	±2.0	±0.2
		50 µL	±1.0	±0.5	±0.8	±0.4
		100 µL	±0.8	±0.8	±0.3	±0.3
30 µL – 300 µL	Orange 20 µL – 300 µL 55 mm	30 µL	±3.0	±0.9	±1.0	±0.3
		150 µL	±1.0	±1.5	±0.5	±0.75
		300 µL	±0.6	±1.8	±0.3	±0.9

7.3 Reference 2 fixed

Reference 2 fixed volume

Model	Test tip epT.I.P.S. color code Volume range Length	Eppendorf AG error limits			
		Error			
		Systematic error		Random error	
		± %	± μL	± %	± μL
1 µL	Dark gray 0.1 µL – 10 µL 34 mm	±2.5	±0.025	±1.8	±0.018
2 µL		±2.0	±0.04	±1.2	±0.024
5 µL	Medium gray 0.1 µL – 20 µL 40 mm	±1.2	±0.06	±0.6	±0.03
10 µL		±1.0	±0.1	±0.5	±0.05
20 µL	Light gray 0.5 µL – 20 µL L 46 mm	±0.8	±0.16	±0.3	±0.06
10 µL	Yellow 2 µL – 200 µL 53 mm	±1.2	±0.12	±0.6	±0.06
20 µL		±1.0	±0.2	±0.3	±0.06
25 µL		±1.0	±0.25	±0.3	±0.075
50 µL		±0.7	±0.35	±0.3	±0.15
100 µL		±0.6	±0.6	±0.2	±0.2
200 µL		±0.6	±1.2	±0.2	±0.4
200 µL	Blue 50 µL – 1000 µL 71 mm	±0.6	±1.2	±0.2	±0.4
250 µL		±0.6	±1.5	±0.2	±0.5
500 µL		±0.6	±3.0	±0.2	±1.0
1000 µL		±0.6	±6.0	±0.2	±2.0

Fixed-volume Reference 2

Model	Test tip epT.I.P.S. color code Volume range Length	Eppendorf AG error limits			
		Error			
		Systematic error		Random error	
		± %	± mL	± %	± mL
2.0 mL	Red 0.5 mL – 2.5 mL 115 mm	±0.6	±0.012	±0.2	±0.004
2.5 mL		±0.6	±0.015	±0.2	±0.005

7.4 Test conditions

Test conditions and test evaluation match in compliance with ISO 8655, Part 6. Fine balance with evaporation protection inspected by the office of weights and measures

- Number of determinations: 10
- Use of water in accordance with ISO 3696
- Inspection at 20 °C – 27 °C ±0.5 °C
- Dispensing on the tube wall

7.5 Ambient conditions

	Temperature range	Relative humidity
Storage without transport packaging	-5 °C — 45 °C	10 % — 95 %
Operating conditions	5 °C — 40 °C	10 % — 95 %

Technical specifications subject to change.

8 Ordering Information

8.1 Single-channel pipettes, variable volume

Order no. (International)	Order no. (North America)	Description
Eppendorf Reference 2, variable		
4920 000.016	4920000016	Single-channel; serial number on upper and lower part dark gray, 0.1 - 2.5 µL
4920 000.024	4920000024	medium gray, 0.5 - 10 µL
4920 000.032	4920000032	light gray, 2 - 20 µL
4920 000.040	4920000040	yellow, 2 - 20 µL
4920 000.059	4920000059	yellow, 10 - 100 µL
4920 000.067	4920000067	yellow, 20 - 200 µL
4920 000.075	4920000075	orange, 30 - 300 µL
4920 000.083	4920000083	blue, 100 - 1 000 µL
4920 000.091	4920000091	red, 0.25 - 2.5 mL
4920 000.105	4920000105	violet, 0.5 - 5 mL
4920 000.113	4920000113	turquoise, 1 - 10 mL

8.2 Single-channel pipettes, fixed volume

Order no. (International)	Order no. (North America)	Description
Eppendorf Reference 2, fixed		
4921 000.010	4921000010	Single-channel; serial number on upper and lower part dark gray, 1 µL
4921 000.028	4921000028	dark gray, 2 µL
4921 000.036	4921000036	medium gray, 5 µL
4921 000.044	4921000044	medium gray, 10 µL
4921 000.052	4921000052	yellow, 10 µL
4921 000.060	4921000060	light gray, 20 µL
4921 000.079	4921000079	yellow, 20 µL
4921 000.087	4921000087	yellow, 25 µL
4921 000.095	4921000095	yellow, 50 µL
4921 000.109	4921000109	yellow, 100 µL
4921 000.117	4921000117	yellow, 200 µL
4921 000.125	4921000125	blue, 200 µL
4921 000.133	4921000133	blue, 250 µL
4921 000.141	4921000141	blue, 500 µL
4921 000.150	4921000150	blue, 1000 µL
4921 000.168	4921000168	red, 2 mL
4921 000.176	4921000176	red, 2.5 mL

Ordering Information

Eppendorf Reference® 2
English (EN)

8.3 Single-channel lower part for variable volume and fixed volume

Order no. (International)	Order no. (North America)	Description
		Eppendorf Reference 2 lower part Single-channel; lower part with separate serial number 2.5 µL, dark gray, variable: 0.1 µL - 2.5 µL, fixed: 1 µL, 2 µL 10 µL, medium gray, variable: 0.5 µL - 10 µL, fixed: 5 µL, 10 µL
4920 601.001	4920601001	20 µL, light gray, variable: 2 µL - 20 µL, fixed: 20 µL
4920 602.008	4920602008	20 µL, yellow, variable: 2 µL - 20 µL, fixed: 10 µL, 20 µL
4920 603.004	4920603004	100 µL, yellow, variable: 10 - 100 µL, fixed: 25 µL, 50 µL, 100 µL
4920 604.000	4920604000	200 µL, yellow, variable: 20 - 200 µL, fixed: 200 µL
4920 605.007	4920605007	300 µL, orange, variable: 30 - 300 µL
4920 606.003	4920606003	1 000 µL, blue, variable: 100 - 1 000 µL, fixed: 200 µL, 250 µL, 500 µL, 1 000 µL
4920 607.000	4920607000	2.5 mL, red, variable: 0.25 - 2.5 mL, fixed: 2 mL, 2.5 mL
4920 608.006	4920608006	5 mL, violet, variable: 0.5 - 5 mL
4920 609.002	4920609002	10 mL, turquoise, variable: 1 - 10 mL
4920 610.000	4920610000	
4920 611.007	4920611007	

8.4 Multi-channel pipettes

Order no. (International)	Order no. (North America)	Description
		Eppendorf Reference 2, variable 8-channel; serial number on upper and lower part medium gray, 0.5 - 10 µL
4922 000.013	4922000013	yellow, 10 - 100 µL
4922 000.030	4922000030	orange, 30 - 300 µL
		Eppendorf Reference 2, variable 12-channel; serial number on upper and lower part medium gray, 0.5 - 10 µL
4922 000.021	4922000021	yellow, 10 - 100 µL
4922 000.048	4922000048	orange, 30 - 300 µL
4922 000.064	4922000064	

8.5 Multi-channel lower parts

8.5.1 8-channel lower parts

Order no. (International)	Order no. (North America)	Description
		Eppendorf Reference 2 lower part 8-channel; lower part with separate serial number medium gray, 0.5 - 10 µL
4922 601.009	4922601009	yellow, 10 - 100 µL
4922 603.001	4922603001	orange, 30 - 300 µL

8.5.2 12-channel lower parts

Order no. (International)	Order no. (North America)	Description
		Eppendorf Reference 2 lower part 12-channel; lower part with separate serial number medium gray, 0.5 - 10 µL
4922 602.005	4922602005	yellow, 10 - 100 µL
4922 604.008	4922604008	orange, 30 - 300 µL

8.6 Spare parts, accessories and pipette tips



The ordering information can be found on the Reference 2 CD.

Ordering Information
Eppendorf Reference® 2
English (EN)

9 Patents

U.S. Patent No.	7,434,484	Is used by Reference 2 pipettes with variable volume setting (single-channel and multi-channel) and fixed volume with following nominal volumes:	1 µL, 2 µL, 2.5 µL, 5 µL, 10 µL, 20 µL, 25 µL, 50 µL, 100 µL, 200 µL, 250 µL, 300 µL, 500 µL and 1000 µL
U.S. Patent No.	7,673,532	Is used by Reference 2 pipettes with variable volume setting (multi-channel) with following nominal volumes:	10 µL, 100 µL, 300 µL
U.S. Patent No.	7,674,432	Is used by Reference 2 pipettes with variable volume setting (single-channel and multi-channel) and fixed volume with following nominal volumes:	1 µL, 2 µL, 2.5 µL, 5 µL, 10 µL, 20 µL, 25 µL, 50 µL, 100 µL, 200 µL, 250 µL, 300 µL, 500 µL, 1000 µL, 1 mL, 2 mL, 2.5 mL, 5 mL and 10 mL
U.S. Patent No.	8,297,134	is used by Reference 2 pipettes with variable volume setting (single-channel and multi-channel) and fixed volume with following nominal volumes:	1 µL, 2 µL, 2.5 µL, 5 µL, 10 µL, 20 µL, 25 µL, 50 µL, 100 µL, 200 µL, 250 µL, 300 µL, 500 µL, 1000 µL, 1 mL, 2 mL, 2.5 mL, 5 mL and 10 mL

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